Platinum™ SuperFi™ DNA Polymerase for the highest success in PCR

Rasa Sukackaitė, Martyna Simutytė, Skaistė Valinskytė, Laurynas Vanagas, Karolis Matjošaitis, Renata Rimšelienė, Remigijus Skirgaila. Thermo Fisher Scientific Baltics UAB, V. A. Graičiūno 8, Vilnius, LT-02241 Lithuania

ABSTRACT

Platinum ™ SuperFi ™ DNA Polymerase is a new proofreading DNA polymerase, which combines superior fidelity with Platinum ™ hot-start technology. Featuring >100X *Taq* fidelity, Platinum SuperFi DNA Polymerase is ideally suited for cloning, mutagenesis, template generation for sequencing and other applications requiring high accuracy. The product is based on new technologies providing top fidelity, specificity and yield: engineered DNA polymerase, Platinum hot-start technology and GC enhancer. The Platinum SuperFi DNA Polymerase was engineered by mutagenesis and fusion of a DNA-binding domain for extremely low error rate and high processivity. High processivity enables fast cycling protocols, amplification of long targets and increased resistance to inhibitors. The hot-start technology is based on monoclonal antibodies that efficiently inhibit enzyme activity until the initial PCR denaturation step. Inhibition of both polymerase and proofreading nuclease activities prevents non-specific amplification and primer degradation resulting in a greater yield of the target amplicon with the added convenience of room-temperature PCR setup. The formulation of the GC enhancer, which is supplied in a separate vial with the Platinum SuperFi DNA Polymerase products, was developed by extensive screening of PCR additives and their combinations for specific amplification and improved yields of targets with high-GC content. Platinum SuperFi DNA Polymerase shows leading performance in all categories important to the highest success in PCR: fidelity, specificity, yield, sensitivity and amplification of difficult targets (long, GC rich, in presence of inhibitors).

INTRODUCTION

Accurate amplification without sequence errors and non-targeted bands is extremely important for cloning, site specific mutagenesis, template generation for sequencing and other applications. Here we present results from a new high fidelity hot-start DNA polymerase.

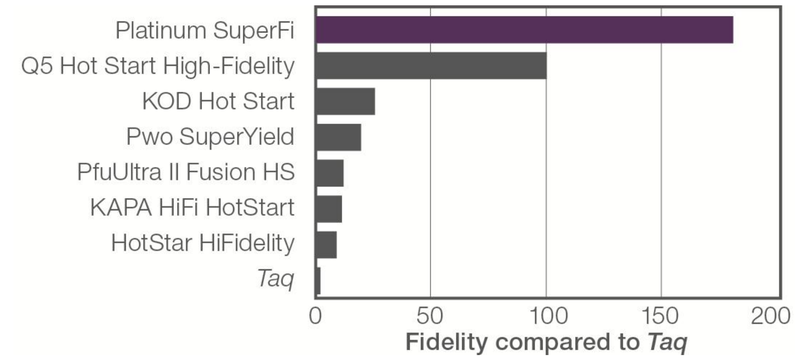
- Invitrogen™ Platinum™ SuperFi™ DNA Polymerase
- Invitrogen™ Platinum™ SuperFi™ Green DNA Polymerase
- Invitrogen™ Platinum™ SuperFi™ PCR Master Mix
- Invitrogen™ Platinum™ SuperFi™ Green PCR Master Mix

RESULTS

Platinum SuperFi DNA Polymerase is based on new technologies:

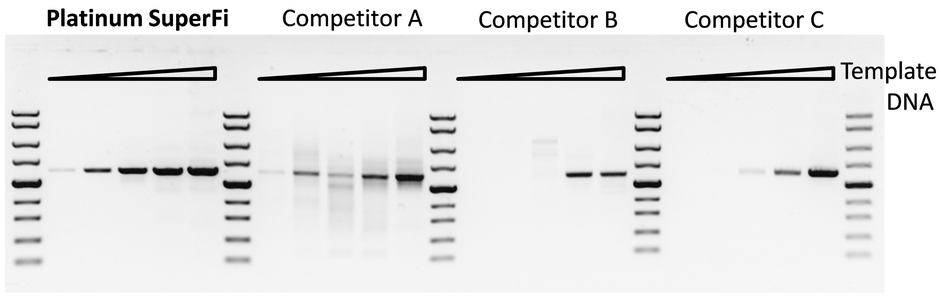
- Engineered polymerase for fidelity, processivity and inhibitor resistance
- Platinum hot-start technology for specificity and yield: complete inhibition of polymerase and proofreading activities at low temperatures
- Optimized GC enhancer for difficult sequences.

Figure 1. Leading fidelity determined by NGS



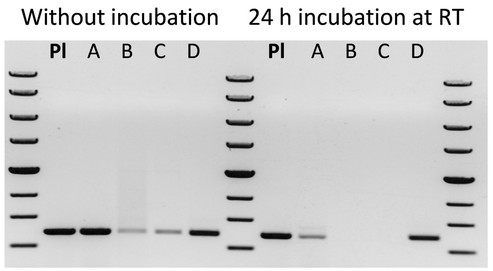
Relative fidelity values of different DNA polymerases. Polymerase fidelity was measured by next-generation sequencing. The background level of experimental errors was estimated from PCR-free library sequencing data. Due to the very low error rates associated with the high-fidelity enzymes, it is challenging to quantify statistically significant differences; however, reactions were run in parallel and polymerase fidelities normalized to *Taq* polymerase. Error rates corresponding >100x Taq are extremely close to the background and cannot be determined in a statistically significant manner.

Figure 2. High yield even from lowest template amounts



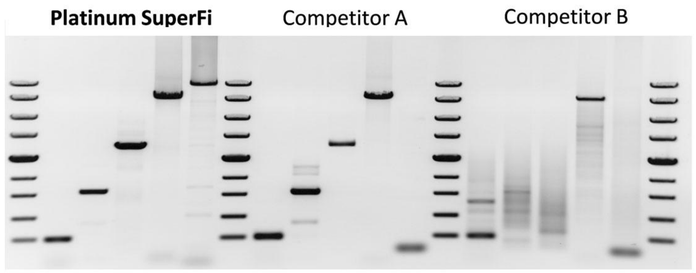
High sensitivity and reliable amplification from low amounts of input DNA. Amplification of 2 kb fragment from 0.4, 2, 10, 50, and 250 ng human gDNA in 50 µL PCR reactions using Platinum SuperFi DNA Polymerase and competitor DNA polymerases (A—KOD Hot Start, B—*PfuUltra II* Fusion Hot Start, and C—KAPA HiFi HotStart PCR Kit).

Figure 3. Stability of PCR reactions



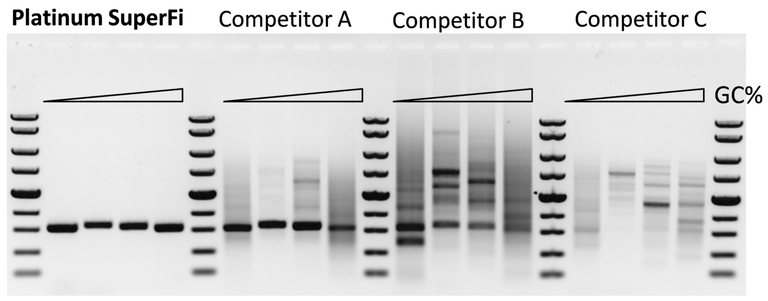
Platinum SuperFi DNA Polymerase stability at room temperature. PCR reactions were set up and incubated at room temperature 0 and 24 h before loading in the thermal cycler. Amplification was performed using Platinum SuperFi (lane Pl), NEB Q5 Hot Start (A), Takara PrimeSTAR HS (B); KAPA HotStart PCR Kit (C); Merck KOD Hot Start (D).

Figure 4. Amplification of targets up 20 kb



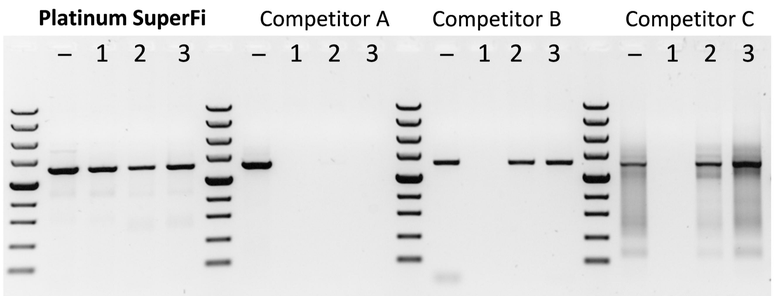
Versatility across a broad range of amplicon lengths. Amplification of 200 bp, 700 bp, 2 kb, 7.5 kb and 20 kb targets from human genomic DNA template. A - KAPA HotStart PCR Kit; B - Merck KOD Hot Start.

Figure 5. Amplification of GC rich targets



Robust amplification of GC-rich DNA. Amplification of ~700 bp GC rich targets (66%, 69%, 73%, 76% GC) from human genomic DNA template using enhancers if provided. A - Merck KOD Hot Start; B - Takara PrimeSTAR GXL; C - Agilent PfuUltra II Fusion Hot Start.

Figure 6. Resistance to PCR inhibitors



Resistance to inhibitors. Amplification of a 2 kb target from human genomic DNA template in presence of common PCR inhibitors. – no inhibitor; 1 – heparin, 0.15 µg/µl; 2 - xylan, 0.5 µg/µl; 3 – humic acid, 0.5 ng/µl. A – NEB Q5 Hot Start; B - KAPA HotStart PCR Kit; C - Merck KOD Hot Start.

CONCLUSIONS

Platinum SuperFi DNA Polymerase shows leading performance for the highest success in PCR.

- **Fidelity**: minimal sequence error rate
- **Specificity**: minimum non-targeted bands even after incubation of PCR reaction at room temperature
- **Yield**: high yield even from low template amounts
- **Difficult targets**: long, GC rich, in presence of inhibitors
- **Convenience**: room temperature set up, 2X master mix, fast cycling, direct gel loading.

ACKNOWLEDGEMENTS

Vilija Drėgūnienė, Greta Daujotaitė, Stephanie Leisso, Eimantas Astromskas, Milda Kaniušaitė, Jurgita Rubekina, Arūnas Lagunavičius, Dovilė Cicėnaitė, Šarūnas Streckis, Vilius Bešėnas, Agnė Mazūrienė, Tatjana Kavaliūnienė, Deividas Jasulevičius, Nijolė Razmienė, Lina Šmigelskienė, Birutė Kaziliūnienė, Laura Paliukonienė, Dovilė Rekertienė, Gražina Mikšytė, Agnė Bujanauskaitė, Bernadette Perfect, Eglė Balčiūnaitė, Violeta Popendikytė, Gediminas Alzbutas

TRADEMARKS

© 2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Q5 is a trademark of New England BioLabs, Inc. Pwo and Expand are trademarks of Roche Molecular Systems Inc. PfuUltra and Herculase are trademark of Agilent, Inc. KAPA HiFi HotStart is a trademark of KAPA Biosystems, Inc. HotStar HiFidelity is a trademark of Qiagen, Inc. PrimeSTAR and Advantage are trademarks of Takara-Clontech Laboratories, Inc.